REMARKS

Claims 25, 26 and 28-32 are pending. Claim 27 has been cancelled, and claims 25, 26 and 32 have been amended for clarity. Claims 33-48 have been withdrawn as being directed to a non-elected invention. Applicants reserve the right to pursue these and other claims in continuations and divisionals. Applicants respectfully submit that no new matter has been introduced.

In the Office Action dated April 29, 2009, claims 25-32 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement.

Applicants note the Examiner's argument set forth on pages 3-4 of the Office Action, but in view of amendments made to the claims, particularly independent claim 25, believes that the § 112, first paragraph, rejection has been overcome. Applicants respectfully point out that support for the limitation "up to 80 mM" is set forth in several places in the original specification, as noted by the Examiner, e.g., page 7, paragraph 25, and thus does not constitute new matter.

Reconsideration and withdrawal of the § 112, first paragraph, rejection of claims 25-32 are respectfully requested.

Claims 25-32 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite.

Again, Applicants note the Examiner's argument set forth on pages 4-6 that the claims are also indefinite. As above, Applicants respectfully submit that, in view of the claim amendments and arguments set forth herein, the present invention, as claimed, is definite. Claim 32 is also amended for clarity.

Reconsideration and withdrawal of the § 112, second paragraph, rejection of claims 25-32 are respectfully requested.

Claims 25-32 are rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,86,1270 to Nelis, in view of a newly-cited *Proceedings of the National Academy of Science* article by Kuroda et al. ("Kuroda").

The induction solution of the present invention facilitates the rapid detection of coliform bacteria, particularly the detection of coliform and like bacteria in the absence of cell growth. This result is obtained by greatly increasing the natural synthesis of the enzymes β-galactosidase and/or β-glucuronidase, through an unexpected combined inductive action between some common synthetic inducers (isopropyl-β-D-thiogalactopyranoside IPTG and/or methyl-β-D-glucuronide) and a pool of natural levorotatory amino acids.

As set forth in more detail in the specification, tryptophan, leucine and isoleucine were important ingredients, while the simultaneous absence of methionine and threonine strongly reduced the inducing effect. As noted in the specification, in general, a proportioned combination of amino acids gave good results. All other components are present in a minimal amount only to allow survival of the cells and expression of the enzyme activity. Indeed, Applicants discovered that microbial growth is strongly prevented, due to the scarcity of ingredients contained in the original "induction solution". Applicants respectfully submit that this is the first time that a no-growth medium, presenting a fully and clearly defined composition, is employed to face the problem of detecting a very limited amount of coliforms/E. coli and like bacteria in a sample in such a short time. Applicants discovered that the selectivity of the claimed action is actually guaranteed from the induction of specific enzymes.

With reference now to Nelis, this reference expressly describes "a growth medium containing nutrients to support propagation of the bacteria" called "improved luminescence medium (ILM)". The latter contains, among other things, a carbon source (sugar or polyalcohol), nitrogen sources (tryptone and monoammonium phosphate) and bile salts. The entire purpose here is to grow target bacteria on a membrane filter, where enzyme induction occurs "in the course of their growth and metabolism." Indeed, the "improved luminescence medium" set forth in Nelis is a growth medium, whereas the "induction solution" claimed by the

Applicant aims to an enzyme induction in the absence of cell proliferation, as claimed. Whereas Nelis and like references detect coliforms and such by virtue of their growth or cell proliferation, the present invention does not rely on cell growth and operates quite differently.

Accordingly, Applicants respectfully submit that Nelis is a flawed primary reference, and fails to provide a framework for an obviousness rejection.

Newly-cited Kuroda, added to cure the deficiencies of the primary reference, generally describes the induction of beta-galactosidase (i.e., lacZ expression) by the nutritional downshift in the wild type E. coli (in particular, see page 14266, right column, last paragraph) and that the availability of free amino acids is important for enzyme production (i.e., beta-galactosidase) after the nutritional downshift (in particular, see page 14267, right column, 1st paragraph).

However, Applicants respectfully submit that there is an extremely important point that must be noted here: the whole of Kuroda's investigation concerns nutritional downshifts from rich 2 x YT medium to minimal MOPS medium. This means that the abovementioned induction of lacZ is described only in conditions of amino acids supplemented to the MOPS minimal growth medium. Additionally, it is clearly indicated that the amino acids also cause remarkable reduction of growth lag and thus they are added in Kuroda in order to support cell growth recovery. This is in complete contrast with the induction solution claimed herein by the Applicants, which targets the use of amino acids for the expression of inducible enzymes in the absence of cell growth.

Accordingly, Applicants respectfully submit that Kuroda, too, is deficient, and fails to cure the aforementioned deficiencies of the primary reference. Applicants respectfully submit that the combination of Nelis with Kuroda fails to render the present invention, as claimed, obvious. Applicants also respectfully disagree with the Examiner's interpretation set forth in the Action.

In any event, based on the aforementioned, Applicants will further demonstrate that it would not be obvious to a person skilled in the art to combine Nelis and Kuroda in the manner proposed to render the present invention obvious.

For example, the growth medium described by Kuroda is only apparently complementary to the ILM set forth in Nelis. By analyzing in depth the differences and similarities between these two references, Applicants respectfully submit that it is possible to demonstrate not only that said media of Kuroda and Nelis are equivalent in both purpose and nutrient composition, but also that they are quite different from the claimed induction solution of the present invention in both purpose and nutrient composition.

As noted by the Examiner, "Kuroda et al discloses an induction solution (suitable for rapid detection of coliform cells, capable of inducing the expression of inducible enzymes, beta-glucuronidase and betagalactosidase, in the absence of cell growth) comprising at least one amino acid or a mixture of amino acids." Applicants respectfully disagree.

Applicants, respectfully submit that the absence of cell growth is in no way described or suggested by Kuroda. In fact, all Kuroda demonstrates is that the presence of amino acids in combination with MOPS accelerates E. coli growth recovery from the nutritional downshifts (Fig. 3 shows that supplementation of amino acids to the MOPS medium abolishes the growth lag). The use of amino acids in the absence of other nutritional components is not contemplated, not disclosed and not suggested.

This is better understandable when comparing the composition of Nelis, Kuroda and the claimed induction solution in terms of nutrient supply. Set forth below is a table to better differentiate Nelis and Kuroda from the present invention.

Table: Media composition in terms of nutrient supply

Nutrient source	Nelis (ILM)	Kuroda	Applicant

Carbon source	Sugar (maltose) or Polyalcohol (mannitol)	Sugar (glucose 22 mM)	None
Nitrogen source	Ammonium (tryptone and monoammonium phosphate)	Ammonium (ammonium chloride 4 mM)	None
Amino acids	Protein hydrolysate (tryptone / yeast)	Purified	Purified
Minerals	Not mentioned	All those supporting growth (including trace metals)	None

The table clearly indicates that the medium used by Kuroda is much more effective than Nelis' ILM in promoting bacterial growth, due to the presence of specific mineral growth-supporting components. Being a selective medium, Nelis needs to discourage growth of non-target bacteria, whereas MOPS minimal medium is known for being among the most complete and effective minimal media.

By way of background, the below abstract of the original article introducing MOPS by Frederick C. Neidhardt, Philip L. Bloch, and David F. Smith (Culture Medium For Enterobacteria, 1974, Journal of Bacteriology, Vol. 119, N. 3, p.736-747) further illustrates the many differences of Nelis and Kuroda from the innovation presently claimed. As noted in the abstract:

> *"A new minimal medium for Enterobacteria has been developed. It supports growth of Escherichia coil and Salmonella typhimurium at rates comparable to those of any of the traditional media that have high phosphate concentrations, but each of the macronutrients (phosphate, sulfate, and nitrogen) is present at a sufficiently low level to permit isotopic labeling. Buffering capacity is provided by an organic dipolar ion, morpholinopropane sulfonate, which has a desirable pK* (7.2) *and no apparent inhibitory effect on growth. The medium has been developed with the objectives of (I) providing reproducibility of chemical composition, (ii) meeting the experimentally determined nutritional needs of the cell, (iii) avoiding an unnecessary excess of the major ionic species, (iv) facilitating the adjustment of the*

> *levels of individual ionic species, both for isotopic labeling and for nutritional studies, (v) supplying a complete array of micronutrients, (vi) setting a particular ion as the crop-limiting factor when the carbon and energy source is in excess, and (vii) providing maximal convenience in the manufacture and storage of the medium."*

According to the definition set forth hereinabove, MOPS is not just a buffer system, but a complete growth medium. A copy of this article is included in an Information Disclosure Statement filed concurrently herewith.

Even if it is true that Kuroda discloses that, after a nutritional downshift, the presence of amino acids is necessary for inducing the expression of inducible enzymes, beta-glucuronidase and beta-galactosidase, this in no way renders the present invention obvious. Indeed, since this medium accelerates the recovery of E. coli reproduction potential, remarkable differences exist between the medium used by Kuroda in their investigations and the induction solution claimed by the Applicants.

Applicants submit that the claimed mixture of amino acids is not only necessary for inducing the expression of inducible enzymes, beta-glucuronidase and beta-galactosidase after a nutritional downshift, but it is useful, in terms of nutritional potential, for inducing the expression of inducible enzymes, beta-glucuronidase and betagalactosidase in the absence of cell growth, as presently claimed. Since Kuroda always tested the amino acids in combination with MOPS, the concept of using amino acids in a "no growth" contest is neither taught nor suggested by the general writings of Kuroda.

Applicants respectfully submit that the disclosure of Nelis and the paper of Kuroda lead to identical conclusions regarding the use of amino acids with reference to specific induction of inducible enzymes, betaglucuronidase and beta-galactosidase, i.e., the supplementation of amino acids in different forms is necessary for supporting enzyme induction and fostering cell growth.

Applicants further respectfully submit that Kuroda confirms via molecular methods the implicit conclusions concerning the importance of amino acids set forth in the disclosure of Nelis. Applicants respectfully submit that a person skilled in the art, starting from a disclosure aiming at fostering cell growth based on targeting growth selectivity, like in the case of Nelis, would not receive any hint from another disclosure aiming at fostering cell growth based on targeting growth recovery, like in the case of Kuroda, to realize that an induction solution would be able to induce the bacteria detection in the absence of cell growth, as presently claimed.

As expressly set forth hereinabove in connection with both Nelis and Kuroda, the medium composition strongly affects the bacterial metabolism and the cell proliferation deactivates the effect targeted by Applicants in their innovation.

In other words, the induction solution of the present invention, as claimed, forces bacteria to allow only one pathway, the production of the requested enzyme, whereas the MOPS supplemented with amino acids and the "improved luminescence medium" support favourable conditions for duplication and expression of different metabolic pathways, as set forth in Nelis, Kuroda and any combinations thereof. In fact, by the use of the MOPS supplemented with amino acids or the "improved luminescence medium", bacteria are engaged in other demanding metabolic activities and do not produce so rapidly a detectable amount of requested enzyme. The cited references teach away from the claimed invention and utterly fail to anticipate or render obvious the innovation set forth and claimed herein.

In view of the claim amendments and arguments set forth hereinabove, Applicants respectfully submit that claims 25-26 and 28-32 are readily distinguishable from both Nelis and Kuroda, along with the proposed or other combinations thereof. Applicants respectfully submit that the § 103(a) rejection is overcome.

Applicants respectfully request that the § 103(a) rejection be reconsidered and withdrawn at least in view of the aforementioned claim amendments and arguments made.

Reconsideration and withdrawal of all §§ 112 and 103 rejections are respectfully requested.

The references cited by the Examiner but not applied appear of no more relevance than the art cited. Applicants submit herewith an Information Disclosure Statement setting forth additional art that has come to Applicants' attention. Applicants respectfully request that the Examiner consider the references submitted herewith and initial the enclosed PTO-1449 form to substantiate same.

In view of the above amendments and remarks, Applicant respectfully requests a Notice of Allowance. If the Examiner believes a telephone conference would advance the prosecution of this application the Examiner is invited to telephone the undersigned at the below-listed telephone number.

Respectfully submitted,

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